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(54) Title: SCREENING METHOD FOR IDENTIFYING WOMEN AT INCREASED RISK FOR PRETERM DELIVERY

(57) Abstract

The present invention provides an early, biochemical indication of increased risk of preterm delivery. The method comprises obtaining a cervicovaginal secretion sample from a pregnant patient after about week 12 of gestation and determining the level of defensins in the sample. The presence of an elevated defensin level in the sample indicates an increased risk of preterm delivery. The test is a screening assay that can detect women at risk of imminent delivery, as early as two to three weeks prior to delivery.

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SCREENING METHOD FOR IDENTIFYING WOMEN AT INCREASED RISK FOR PRETERM DELIVERY

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to methods for detection of preterm delivery. In particular, this invention is directed to determining an early indication of increased risk of preterm delivery by detecting an increased level of defensins in cervicovaginal secretion samples.

Description of the Prior Art

Determination of impending preterm births is critical for increasing neonatal survival of preterm infants. In particular, preterm neonates account for more than half, and maybe as much as three-quarters of the morbidity and mortality of newborns without congenital anomalies. Although tocolytic agents which can delay delivery were introduced 20 to 30 years ago, there has been only a minor decrease in the incidence of preterm delivery. It has been postulated that the failure to observe a larger reduction in the incidence of preterm births is due to errors in the diagnosis of preterm labor and to the patients' conditions being too advanced for tocolytic agents to successfully delay the birth.

Traditional methods of diagnosis of preterm labor have high false-negative and false-positive error rates [Friedman et al, *Am. J. Obstet. Gynecol.* **104**:544 (1969)]. In addition, traditional methods for determining impending preterm delivery, particularly in patients with clinically intact membranes, may require subjective interpretation, may require sophisticated training or equipment [Garl et al, *Obstet. Gynecol.* **60**:297 (1982)] or may be invasive [Atlay et al, *Am. J. Obstet. Gynecol.* **108**:933 (1970)].

Accordingly, an early, objective biochemical marker indicative of increased risk for preterm delivery was sought.

Recently, Lockwood et al [*New Engl. J. Med.* 325:669-674 (1991)] reported that fetal fibronectin in cervical and vaginal secretions indicates pregnancies which are at risk of imminent delivery. The authors postulate that damage to the fetal membranes may release fetal fibronectin into the cervix and vagina, thus giving rise to the biochemical marker.

Other markers which may be released in women with true threatened pregnancies can be used to screen those women who should be closely monitored and to provide additional information about the stage of the disease.

SUMMARY OF THE INVENTION

The present invention provides an early, biochemical indication of increased risk of impending preterm delivery. The method comprises obtaining a cervicovaginal secretion sample from a pregnant patient after about week 12 of gestation and determining the level of defensins in the sample. The presence of an elevated defensin level relative to that which is characteristic of pregnancies that proceed to term indicates an increased risk of preterm delivery. The test is both a sensitive and specific screen for pregnancies at risk and can detect impending delivery as early as two to three weeks prior to delivery.

The test is preferably administered to women at about 12 weeks gestation and repeated at each prenatal visit (every two to four weeks) until at least week 37, preferably until delivery if the test is negative. For those patients whose assay result indicates an increased risk of preterm delivery, a test of the patient's fetal fibronectin level can be made to confirm the increased risk and to estimate how soon the delivery will be. In

addition, those patients can be carefully monitored, as for other patients at risk.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is a screening assay which provides an early, biochemical indication of increased risk of preterm delivery. The method can provide an indication of impending preterm delivery as early as two to three weeks prior to delivery. The method allows early intervention in the course of preterm delivery and provides an additional factor which can indicate those pregnancies at greatest risk.

The method comprises obtaining a cervicovaginal secretion sample from the vaginal cavity or the external cervical canal from a pregnant patient after about week 12 of pregnancy and prior to about week 36 or 37 and determining the level of defensins in the sample. The presence of an elevated level of the protein in the sample indicates a patient who is at risk for preterm delivery.

The present method can determine impending delivery from early in gestation through week 36 or 37. Deliveries prior to 20 weeks gestation are generally called spontaneous abortions rather than preterm deliveries. The present method can be used to detect spontaneous abortions (12 to 20 weeks gestation) and preterm deliveries (20 to 37 weeks gestation). Term pregnancies are from 37 to 40 weeks.

Defensins

Human defensins, also known as human neutrophil proteins (HNPs), are a class of small proteins (about 29 to 33 amino acid residues per chain) that have antimicrobial, cytotoxic and chemoattractant properties and that are involved in host defense and inflammatory processes. Defensins, which are stored in the azurophil granules of neutrophils, encounter microbes chiefly through the fusion of azurophil granules with phagocytic

vesicles within the neutrophil, whereupon the defensins neutralize ingested microorganisms contained in the vesicles. Release of defensins from neutrophils to the extracellular fluid also occurs.

At least four human defensins have been reported. The three major defensins, HNP-1, HNP-2, and HNP-3, contain 30, 29, and 30 amino acid residues, respectively, are highly cationic, and are nearly identical in amino acid sequence. HNPs 1 and 3 differ from HNP-2 only in that HNPs 1 and 3 begin with an additional N-terminal residue (alanine in the case of HNP-1 and aspartate in the case of HNP-3). The fourth defensin, HNP-4, contains 33 residues and corresponds in amino acid sequence to the first three defensins at only 11 positions. In relation to HNP-2, HNP-4 begins with one additional N-terminal residue (valine) and ends at the C-terminus with three additional residues. The 11 sequence positions at which HNPs 1-4 are identical include six cysteine residues that form intrachain disulfide bonds between cysteines 3 and 31, 5 and 20, and 10 and 30. Whether defensins are monomeric or dimeric is unclear. Selsted et al [*J. Clin. Invest.* 76:1436-1439 (1985)] describes the amino acid sequence of HNPs 1-3, and Wilde et al [*J. Biol. Chem.* 264:11200-11203 (1989)] describes the sequence of HNP-4 and the homology between HNP-4 and HNPs 1-3. Those references are hereby incorporated by reference.

The first three defensins are present in approximately equal amounts and comprise about 99% of the defensins, with HNP-4 comprising the remainder. For purposes of this invention, detection of the sum of HNPs 1-3 is detection of defensins, since the amount of HNP-4 present in the sample is not significant.

Patients to be Tested

The present method can be used on any pregnant woman following about 12 weeks gestation and prior to term

pregnancies (week 36 or 37). In addition to screening any pregnant woman to determine whether delivery is imminent, the patients who are preferably screened are those patients with clinically intact membranes in a high risk category for preterm delivery, and more preferably, all those women whose pregnancies are not sufficiently advanced to ensure delivery of a healthy fetus. Ninety percent of the fetal morbidity and 100 percent of the fetal mortality associated with preterm delivery is for those fetuses delivered prior to 32 to 34 weeks gestation. Therefore, 32 to 34 weeks gestation is an important cutoff for the health of the fetus, and preferably women whose pregnancies are at least about 12 weeks and prior to 34 weeks in gestation are tested.

In addition there are a large number of factors known to be associated with the risk of preterm delivery. Those factors include multiple fetus gestations; incomplete cervix; uterine anomalies; polyhydramnios; nulliparity; previous preterm rupture of membranes or preterm labor; preeclampsia; first trimester vaginal bleeding; little or no antenatal care; and symptoms such as abdominal pain, low backache, passage of cervical mucus and contractions. Any pregnant woman at 12 or more weeks gestation with clinically intact membranes and having one or more risk factors for preterm delivery is preferably tested throughout the risk period; i.e., until about week 34 to 37.

Sample

The sample is obtained in the vicinity of posterior fornix, the ectocervix or external cervical os. The sample generally comprises fluid and particulate solids, and may contain vaginal or cervical mucus and other vaginal or cervical secretions. The sample is preferably removed with a swab having a dacron or other fibrous tip. Alternatively, the sample can be obtained with a suction or lavage device. Calculations to account for any

additional dilution of the samples collected using liquids can be performed as part of the interpretation of the assay procedure.

Following collection, the sample is transferred to a suitable container for storage and transport to a testing laboratory. It is important that the sample be dispersed in a liquid which preserves proteinaceous analytes. The storage and transfer medium should minimize, preferably prevent, decline in the analyte level during storage and transport. A suitable solution for storage and transfer consists of 0.05 M Tris-HCl, pH 7.4; 0.15 M NaCl, 0.02% NaN_3, 1% BSA, 500 Kallikrein Units/ml of aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mM EDTA, and is described in U.S. Patent No. 4,919,889, issued April 24, 1990. Alternatively, Panyutich et al, *J. Immunol. Meth.* 141:149-155 (1991) describes a preferred assay for defensins which uses 20 mM Tris-HCl, 500 mM NaCl, pH 7.5 (TBS) as the storage solution. Those solutions are also suitable as a sample diluent solution and assay buffer.

Because defensins tend to bind non-specifically to surfaces, preferably the solution contains a suitable detergent, such as cetrimonium bromide (hexadecyltrimethylammonium bromide, "CETAB") as described more fully below in the description of the assay.

Alternatively, home and office use devices for immediate processing of the sample can be used. If used, the sample is placed directly in the device and testing is performed within minutes of sample collection. In such cases, the need to stabilize the analyte is minimized and any solution which facilitates performing the assay and is not detrimental to analyte stability can be used.

Preferably, if the samples contain neutrophils, the neutrophils are lysed and the total defensins in the sample including that released by the lysis is quantitated. The lymphocytes can be lysed by well known methods such as freezing the sample. Preferably, the sample is sufficiently diluted to prevent defensins from

complexing with proteins such as α_2-macroglobulin, interleukins, and transforming growth factor. A dilution that eliminates complexing with proteins in blood samples, 1:1,000, is sufficient. (See Panyutich et al, *J. Immunol. Meth.* **141**:149-155 (1991) which describes a preferred assay for defensins which is performed on blood samples.)

Assay Procedure

Defensins are assayed by any procedure that can determine the presence of a threshold quantity of defensins in the sample. Immunoassays are preferred. Given the similarity of the amino acid sequences of HNP-1, HNP-2, and HNP-3, most antibody compositions are capable of detecting HNP-1, HNP-2, and HNP-3 with comparable sensitivity and are suitable to detect a threshold level of defensins for the purposes of the present invention.

Although antibodies that bind HNP-1, HNP-2 and HNP-3 are not likely to bind HNP-4 due to the differences in the amino acid sequences, failure to quantitate HNP-4 will not effect the assay result. The antibody affinity required for detection of defensins using a particular immunoassay method will not differ from that required to detect other polypeptide analytes.

Anti-defensin antibodies can be produced by a number of methods and can be polyclonal or monoclonal. Polyclonal antibodies can be induced by administering an immunogenic composition comprising a defensin (or defensins) to a host animal. Methods to prepare immunogenic compositions of defensins may vary depending on the host animal and are well known. For example, a defensin or an antigenic portion thereof can be conjugated to an immunogenic substance such as KLH or BSA or provided in an adjuvant or the like. The induced polyclonal antibodies can be tested to determine whether the composition is defensin-specific.

If a polyclonal antibody composition does not provide the desired specificity, the antibodies can be purified to

enhance specificity by a variety of conventional methods. For example, the composition can be purified to reduce binding to other substances by contacting the composition with defensins affixed to a solid substrate. Those antibodies which bind to the substrate are retained. Antibody purification techniques using antigens affixed to a variety of solid substrates such as affinity chromatography materials including SEPHADEX, SEPHAROSE, and the like are well known. In addition, methods to purify HNP-1, HNP-2, and HNP-3 [Ganz et al, *J. Clin. Invest.* **76**:1427-1435 (1985)] and HNP-4 [Wilde et al, *J. Biol. Chem.* **264**:11200-11203 (1989)] are known. Purified defensins can be used to prepare an immunogen, to evaluate antibody specificity, or as a standard or competitive analyte in an assay. The above references are hereby incorporated by reference.

Defensin-specific monoclonal antibodies can also be prepared by conventional methods. A mouse can be injected with an immunogenic composition comprising a defensin(s) or antigenic portion thereof, and spleen cells obtained. Those spleen cells can be fused with a fusion partner to prepare hybridomas. Antibodies secreted by the hybridomas can be screened to select antibodies that react with a defensin and exhibit substantially no reaction with the other proteins which may be present in a sample. Hybridomas that produce antibodies of the desired specificity are cultured by standard techniques. Hybridoma preparation techniques and culture methods are well known and constitute no part of the present invention.

An exemplary preparation of defensin-specific monoclonal antibodies induced using HNP-1 is described in the Examples. Antibody preparation and purification methods are described in a number of publications including Tijssen, P. Laboratory Techniques in Biochemistry and Molecular Biology: Practice and Theories of Enzyme Immunoassays New York: Elsevier (1985), for example.

A number of different types of immunoassays are well known using a variety of protocols and labels. The assay conditions and reagents may be any of a variety found in the prior art. The assay can be heterogeneous or homogeneous, and conveniently, a sandwich assay.

The assay usually employs solid phase-affixed anti-defensin antibodies. The antibodies can be polyclonal or monoclonal. The solid phase-affixed antibodies are combined with the sample. Binding between the antibodies and sample can be determined in a number of ways. Complex formation can be determined by use of soluble antibodies specific for defensin. Alternatively, the capture antibody in a sandwich assay can be an irrelevant antibody. (An irrelevant antibody is an antibody that is specific for an antigen other than defensin.) Panyutich et al [*J. Immunol. Meth.* **141**:149-155 (1991)] reported that the capture (solid phase-affixed) antibody, but not the soluble, biotinylated antibody, can be substituted with a non-HNP-specific antibody with minor loss of sensitivity in the assay and that the same antibody can be used for both the capture and soluble antibodies. The authors suggested that defensins may have an antigenic site recognized by defensin-specific antibodies and an immunoglobulin-binding site distinct from the antigenic site.

The soluble antibodies can be labeled directly or can be detected using labeled second antibodies specific for the species of the soluble antibodies. Various labels include radionuclides, enzymes, fluorescers, colloidal metals and the like. Conveniently, the assay is a quantitative enzyme-linked immunosorbent assay (ELISA) in which monoclonal antibodies specific for defensin are used as both the solid phase-affixed and the enzyme-labeled soluble antibodies.

A preferred sandwich ELISA immunoassay to quantitate human defensins can be performed as described in Panyutich et al [*J. Immunol. Meth.* **141**:149-155 (1991)] and

references therein, the entire contents of which are hereby incorporated by reference. In this assay, defensins in the sample are captured and detected using different monoclonal antibodies for the solid phase-affixed antibody and the soluble (biotinylated) antibody. The antibodies bind HNP-1, HNP-2 and HNP-3 with comparable affinities. Bound defensins are quantitated using avidin peroxidase, which binds to the biotinylated antibodies.

Alternatively, the assay can be based on competitive inhibition, where defensins in the sample compete with a known amount of defensin for a predetermined amount of anti-defensin antibody. For example, any defensin present in the sample can compete with a known amount of the labeled defensin or defensin analogue for antibody binding sites. The amount of labeled defensin affixed to the solid phase or remaining in solution can then be determined.

In another preferred embodiment, the assay is a homogeneous immunoassay in which defensin-specific antibodies are used as the solid phase-affixed and colloidal metal-labeled, soluble antibodies. Alternatively the solid phase-affixed antibodies can be an irrelevant antibody. Appropriate dilution of the conjugate (labeled soluble antibody) can be performed to detect the selected threshold level of defensin as a positive sample.

HNP-1, HNP-2, and HNP-3 have a tendency to bind non-specifically to surfaces. This non-specific binding can be minimized by addition of a suitable detergent, preferably a cationic detergent; e.g., cetrimonium bromide (hexadecyltrimethylammonium bromide, "CETAB"; Sigma Chemical Co.) at a concentration of from about 0.001% to 0.1%, preferably about 0.01% (w/v). Therefore, preferably the assay medium contains a suitable detergent. As discussed previously, the assay medium is preferably also used as the collection medium.

An exemplary assay for the detection of defensins HNP-1, HNP-2, and HNP-3 in cervicovaginal samples is provided in the examples.

Interpretation of Result

A defensin level that is significantly higher than the level that is characteristic of a pregnancy that will proceed to term indicates increased risk of preterm delivery. Preferably, the threshold level that separates risk from non-risk cases is two standard deviations above the average level for pregnancies that proceed to term. A preferred threshold is the amount of defensins in 10^3 polymorphonuclear lymphocytes (about 5 ng).

If the test result is positive (the defensin level is above the threshold value), the patient is preferably tested for the presence of fetal fibronectin in her cervicovaginal secretions. If fetal fibronectin is present in the secretions, the patient is likely to deliver in two to three days. Measures to determine or enhance fetal lung maturity can be undertaken. If the fetal fibronectin assay is negative, the patient should be carefully monitored and repeated evaluations of the patient's fetal fibronectin levels should be performed on subsequent visits. In general, patients at risk for preterm delivery are examined every two weeks from about 22 to 36 weeks, rather than every four weeks as for patients in a low risk category.

If the test is negative (the defensin level is below the threshold), the test is preferably repeated on each subsequent antenatal visit until either the test is positive or the patient reaches term.

This invention is further illustrated by the following specific but non-limiting examples. Temperatures are given in degrees Centigrade and concentrations as weight percent unless otherwise specified. Procedures which are constructively reduced to practice are described in the present tense, and

procedures which have been carried out in the laboratory are set forth in the past tense.

EXAMPLE 1

Preparation of Monoclonal Antibodies Against HNP-1

HNP-1 is purified from human neutrophils as described by Ganz et al [*J. Clin. Invest.* 76:1427-1435 (1985)] and is stored in 0.1% acetic acid at -20°C. Purified HNP-1 is conjugated with bovine serum albumin (BSA), fraction V (Gibco, Paisley, Scotland, U.K.), using glutaraldehyde (Serva, Heidelberg, F.R.G.) in a single-step coupling reaction according to the method of Reichlin [*Meth. Enzymol.* 70:159-165 (1980)].

BALB/c female mice are immunized twice over a 30 day interval with 100 μg of the conjugate, with first injections in complete Freund's adjuvant (Calbiochem, La Jolla, CA). Immune splenocytes and myeloma cells are fused using polyethylene glycol 4000 ("for gas chromatography" Merck, Darmstadt, F.R.G.) as described by Lane et al [*J. Immunol. Methods* 70:34 (1984)]. Fused cells are grown in Hybrimax Iscove's modified Dulbecco's medium (Sigma) containing 10% (v/v) fetal calf serum (Gibco), 10% (v/v) horse serum (Gibco), 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, and 100 U/ml each of penicillin and streptomycin. Hypoxanthine-aminopterin-thymidine and hypoxanthine-thymidine (Sigma) are used for hybridoma selection.

Positive hybridomas are cloned twice over BALB/c spleen cell feeder layers, and the cloned cells are screened by ELISA both for reactivity with HNP-1 conjugated to BSA and also for the absence of reactivity with BSA alone. The isotypes of the monoclonal antibodies from culture supernatants of positive clones are determined by an enzyme immunoassay kit (Biorad, Richmond, CA), using rabbit anti-mouse subclass-specific antibodies.

Large quantities of monoclonal antibodies from the positive clones are prepared by intraperitoneal injection of 2-5 x 10^6 cells from positive clones into BALB/c mice that have been previously primed with 0.5 ml of pristane (Sigma). Monoclonal antibodies from the ascitic fluids are purified by a recombinant protein A purification kit (Beckman, Fullerton, CA). Protein concentrations are determined by the Bradford dye binding assay (Biorad, Richmond, CA) or by absorbance at 280 nm.

The relative affinities of the antibodies for HNP-1 are determined using an ELISA assay in which microtiter plate wells are coated with 1 μg of HNP-1-ovalbumin conjugate, the conjugate having been prepared using ovalbumin (Sigma) and glutaraldehyde in a single-step reaction as above. Purified monoclonal antibodies are then incubated separately in the wells (1 μg per well) and the level of bound monoclonal antibody is determined using the isotype determination kit from Biorad mentioned above. Hybridomas that produce antibodies with the highest reactivity, as determined by ELISA, are cloned twice more.

Biotinylated anti-HNP-1 antibodies are prepared by reaction of antibody (10 mg/ml) with biotin-amidocaproate N-hydroxysuccinimide ester (Sigma) in 0.1 M bicarbonate buffer for 4 hours, followed by dialysis against 0.1 M borate buffer, pH 8.0, containing 0.15 M NaCl.

EXAMPLE 2

Immunoassay to Determine the Defensin Level

The following immunoassay method determines the levels of defensins by quantitating the sum of HNP-1, HNP-2, and HNP-3 in a cervicovaginal sample.

Preparation of Anti-Defensin Antibody-Coated Microtiter Plates

Microtiter plates (NUNC-IMMUNOPLATE I, Nunc, Denmark) are coated with 100 μl of anti-HNP-1 monoclonal antibody (10 μg/ml) in 0.1 M Na_2CO_3 buffer, pH 9.6, at room

temperature overnight. The plates are washed four times in distilled water and then blocked with 200 µl/well of 1% gelatin in 25 mM Tris-HCl, pH 7.5, containing 500 mM NaCl (TBS buffer) for 1 hour at room temperature. The wells are then aspirated to dryness.

HNP Standards

The concentration of a stock solution of purified HNP-1 defensin is established by UV-absorbance measurements at 280 nm. A 1 mg/ml solution of HNP-1 in 0.1% acetic acid containing 0.01% hexadecyltrimethylammonium bromide (CETAB; Sigma) has an absorbance of 3.0. Aliquots are diluted to 1 µg/ml in 0.1% acetic acid and 0.01% CETAB and stored at -20°C. HNP-2 or HNP-3 can be substituted as the standard.

Assay Procedure

The assay is performed as follows. All samples are collected in the vicinity of the posterior fornix or cervical os using a swab containing an absorbent material, e.g. a cotton or dacron swab. Swab samples are immersed in 1.0 ml of sample diluent in a collection vial. The swabs are removed from the solution leaving as such liquid as possible in the collection tube. The samples are filtered through 5 µm pore size polyethylene sample filters (Porex Technologies, Fairburn, Georgia) prior to assay.

Dilutions of standard HNP-1 samples (8, 4, 2, 1, 0.5, 0.25, 0.125 ng/ml final concentration) and samples for HNP measurement are prepared in TBS containing 0.01% CETAB. Duplicate 100 µl aliquots of each sample are placed in separate wells and incubated for 2 hours at room temperature. The samples are then aspirated from the wells, and the wells are washed four times in distilled water. Biotinylated anti-HNP-1 monoclonal antibodies diluted to 0.5 µg/ml in TBS buffer containing 0.01% CETAB

are then added to the wells (100 μL/well) and incubated for 1 hour at room temperature. After aspiration of the samples and four wash steps with distilled water, the wells are incubated for 1 hour with 100 μl of a solution of avidin-peroxidase (Cappel, Westchester, PA) diluted 1/4000 in TBS containing 1% gelatin, followed by aspiration and an additional four washes. The plates are developed by the addition of 100 μl of a reaction mixture containing o-phenylenediamine (0.2 mg/ml; Sigma) in 20 mM citrate buffer, pH 4.7, and 0.25 μl/ml 30% H_2O_2. After 5 minutes at room temperature the reaction is stopped with 50 μl of 2.5 M H_2SO_4 and the absorbance at 492 nm is read by an EIA reader model EL-307 (Biotek, Burlington, VT). Samples with a defensin level above 5 ng indicate that the patient is at risk for a preterm delivery.

EXAMPLE 3

Determining a Pregnancy That is at Increased Risk for Preterm Delivery

A serum sample from a pregnant women at 26 weeks gestation is assayed for defensins according to the procedure described in Example 2. The sample is found to have a defensin level of 10 ng. This value indicates that the woman is at increased risk for preterm delivery.

WHAT IS CLAIMED IS:

1. A method for determining an early indication of increased risk of preterm delivery comprising
   a) obtaining a secretion sample from the vaginal cavity or the cervical canal of a pregnant patient after week 12 and prior to week 37 of pregnancy; and
   b) determining the level of defensins in the sample, an elevated defensins level relative to that which is characteristic of pregnancies that proceed to term indicating an increased risk of preterm delivery.
2. The method of Claim 1 wherein the sample is removed from the posterior fornix.
3. The method of Claim 1 wherein the sample is obtained from the cervical os.
4. The method of Claim 1 wherein the sample obtained from the patient does not have an elevated defensins level and another sample from the patient is assayed for defensins at least two weeks later.
5. The method of Claim 4 wherein a sample from the patient is obtained and assayed for the presence of defensins at intervals of about 4 weeks until the patient delivers or a positive sample is obtained.
6. The method of Claim 1 wherein the sample has an elevated defensins level and a sample from the patient is assayed for the presence of fetal fibronectin.
7. The method of Claim 6 wherein the sample does not contain fetal fibronectin and another sample from the patient is assayed for fetal fibronectin at least one week later.
8. The method of Claim 7 wherein a sample from the patient is obtained and assayed for the presence of fetal fibronectin at two week intervals until the patient delivers or a positive fetal fibronectin

sample is obtained.

9. The method of Claim 1 comprising the steps of
   a) contacting the sample with an anti-defensin antibody for a time sufficient for antigen-antibody binding; and
   b) determining the amount of binding.

10. The method of Claim 9 comprising the steps of
   a) contacting the sample with solid phase-affixed anti-defensin antibody and labeled anti-defensin antibody for a time sufficient for antigen-antibody binding; and
   c) determining the amount of label on the insoluble support.

11. The method of Claim 10 wherein the labeled anti-defensin antibody is conjugated to a label.

12. The method of Claim 11 wherein the label is an enzyme.

13. The method of Claim 10 wherein the anti-defensin antibody is indirectly labeled by reacting the anti-defensin antibody with a labeled antibody specific for the species of the anti-defensin antibody.

14. The method of Claim 1 comprising the steps of
   a) contacting the sample with a solid phase-affixed irrelevant antibody for a time sufficient for antigen-antibody binding and an anti-defensin antibody for a time sufficient for antigen-antibody binding; and
   c) determining the amount of anti-defensin antibody on the insoluble support.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04736

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :G01N 33/53, 33/50, 33/68, 33/551
US CL :436/510, 63, 65
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 436/510, 63, 65, 814

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chem abstracts, Biosis, Medline, CAS registry, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Immunological Methods, Volume 141, issued 1991, A.V. Panyutich et al, "An Enzyme Immunoassay for Human Defensins" pages 149-155, see pages 150-151 and 155.	1-14
Y	American Journal of Obstetrics and Gynecology, Volume 159, issued August 1988, K. Okamura et al, "Nitroblue Tetrazolium Reduction by Leukocytes in the Cervix of Pregnant Women" pages 417-420, see page 419.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04736

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	American Journal of Obstetrics and Gynecology, Volume 165, issued October 1991, R. Romero et al., "Neutrophil Attractant/Activating Peptide-1/Interleukin-8 in Term and Preterm Parturition" pages 813-820, see pages 813 and 815-816.	1-14
Y	US, A, 5,096,830 (Senyei et al) 17 March 1992, see column 4 lines 7-28.	6-8
Y	New England Journal of Medicine, Volume 325, No. 10, issued 05 September 1991, C.J. Lockwood et al, "Fetal Fibronectin in Cervical and Vaginal Secretions as a Predictor of Preterm Delivery" pages 669-674, see page 671.	7-8
Y	E. Harlow et al, "Antibodies: A Laboratory Manual" published 1988 by Cold Spring Harbor Laboratory (N.Y.), pages 342-345 and 561, see pages 342-345 and 561.	13